FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
PU3703USW	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	
10/070633	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/US00/28218	12 October 2000
PRIORITY DATE CLAIMED	
15 October 1999	

TITLE OF INVENTION

METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS

APPLICANT(S) FOR DO/EO/US

ANDERSON, Joanne Elizabeth; TARCZYNSKI, Frank Joseph; WALKER, Dwight Sherod

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

copy of PCT publication cover page
copies of PCT Request and Correction to PCT Request
Power of Attorney (3)
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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070633	PCT/US00/28218	PU3703USW

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	39 - 20 =	19	x $18.00	$342.00
Independent claims	4 - 3 =	1	x $84.00	$84.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
TOTAL OF ABOVE CALCULATIONS =				$1,316.00
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$0.00
SUBTOTAL =				$1,316.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
TOTAL NATIONAL FEE =				$1,316.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00
TOTAL FEES ENCLOSED =				$1,316.00
			Amount to be: refunded	$
			charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **07-1392** in the amount of **$1,316.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1392** A duplicate copy of this sheet is enclosed.

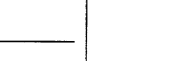
d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23347

PATENT TRADEMARK OFFICE



SIGNATURE

Frank G. Grassler

NAME

31,164

REGISTRATION NUMBER

March 4, 2002

DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: ANDERSON et al.

International Application No. PCT/US00/28218

International Filing Date: 12 October 2000

For: *METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS*

BOX PCT (DO/EO/US)
Commissioner For Patents
Washington, D.C. 20231

FIRST PRELIMINARY AMENDMENT

Sir:

The above-identified application is being transmitted herewith for entry into the U.S. National Phase under Chapter II of the PCT. For the purposes of adding the priority information, please amend the application as follows:

In the Abstract:

Please substitute the attached Abstract, which has been placed on a separate piece of paper according to US practice.

In the Specification:

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. § 371 as a United States National Phase Application of International Application No. PCT/US00/28218 filed October 12, 2000, which claims priority from U.S. Serial No. 60/159,673 filed October 15, 1999 --

REMARKS

Currently claims 1-39 are pending. Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information.

Respectfully submitted,

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Date: March 4, 2002
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METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS

Abstract of the Disclosure

A method for monitoring a solid phase chemical reaction comprises the steps of: *(a)* providing a reaction mixture comprising a solid support and a reaction medium, *(b)* contacting an attenuated total reflection element to said reaction mixture; and then *(c)* monitoring the chemical reaction on the solid support through the attenuated total reflection element. The monitoring step is carried out by attenuated total reflection spectroscopy. An advantage of the invention is that the chemical reaction on the solid support may be directly monitored, rather than indirectly monitoring that chemical reaction by monitoring reaction constituents in the reaction medium.

METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS

Field of the Invention

The present invention concerns methods and instruments for monitoring chemical reactions, and particularly concerns methods and instruments for monitoring solid phase chemical reactions.

Background of the Invention

Traditionally, most chemical reactions for the synthesis of organic compounds have been carried out in liquid phase. However, recent trends in the search for novel chemical and pharmacological agents have concentrated on the preparation of so-called "chemical libraries" as potential sources of new drug candidates. Such chemical libraries are intentionally created collections of differing molecules that can be screened for biological activity. When such libraries are prepared by synthetic chemical reactions, each member of the library is created through a sequential series of chemical reactions, with a different combination of reactions being carried out to create each different member of the library.

To facilitate synthesis and screening of chemical libraries, particularly by automated techniques, different chemical entities within the libraries are often attached to a discrete solid support such as a particle or bead. As a result, a particular compound within that library which is identified as a candidate by the screening technique can be isolated by isolating the discrete solid support to which it is bound.

Chemical synthesis and screening robots for these purposes are known, examples being shown in U.S. Patent No. 5,463,564 to Agrafiotis et al.

Because of the need to construct solid phase combinatorial libraries, reactions that have previously been carried out in liquid phase are now being carried out in

solid phase so that the reaction products are immobilized on a solid support. Solid phase synthesis reactions are typically run for up to 60 hours in order to ensure the reaction goes to completion. The reactions are run for such a long time because making measurements during the reaction required taking a sample and either cleaving the product off of the support or drying the sample. The former approach tends to destroy the product and is time consuming, and the latter approach is time consuming and may degrade the sample. When a large or complex library is desired (as is often the case) the time required for each reaction step significantly slows the construction of the combinatorial library.

In view of the foregoing, there is a need for new techniques for monitoring solid phase synthesis reactions.

Summary of the Invention

The present invention provides a method for monitoring a solid phase chemical reaction. The method comprises the steps of: *(a)* providing a reaction mixture comprising a solid support and a liquid reaction medium, *(b)* contacting an attenuated total reflection element to said reaction mixture; and then *(c)* monitoring the chemical reaction on said solid support through said attenuated total reflection element. The monitoring step is carried out by attenuated total reflection spectroscopy. Advantageously, the chemical reaction on the solid support may be directly monitored, rather than indirectly monitoring that chemical reaction by monitoring reaction constituents in the liquid reaction medium.

The contacting step is carried out under conditions that cause the progression of the desired reaction, with the appropriate reagents included in the reaction medium. The monitoring step is preferably carried out by measuring light absorbance by the reaction mixture, with changing light absorbance being positively associated with the progression of the reaction (*e.g.*, increasing light absorbance being positively associated with the progression of a synthesis reaction, or decreasing light absorbance being positively associated with the progression of a degradation reaction).

The foregoing method can be used to directly monitor the progression of chemical reactions as they proceed on solid phase supports. By measuring the light absorbance of the beads (*e.g.*, infra-red, near infra-red, visible, or ultra-violet light absorbance) attached to the probe, the progression of reaction steps on the bead can be

monitored without having to remove a sample or cleave the molecule off of the bead. While applicable to any type of small or large scale solid phase synthesis, this method is particularly useful for application to solid phase synthesis carried out to generate a solid phase combinatorial library, where the time required for each step to generate the library can be advantageously reduced.

Thus, a further aspect of the present invention is a method of making a combinatorial library preferably comprising at least 100 different compounds, with each of said compounds immobilized on a discrete solid support, by at least two sequential reaction cycles, and with each of said reaction cycles comprising a solid phase chemical reaction, wherein all of the sequential reaction cycles are completed in a total time that averages from not more than 8 or 10 hours for each of the sequential reaction cycles.

The present invention is explained in greater detail in the drawings herein and the specification set forth below.

Brief Description of the Drawings

Figure 1a is a schematic illustration of an apparatus that may be used to carry out the present invention.

Figure 1b is a detailed view of a segment of the apparatus of Figure 1a, showing the adhesion of the solid phase synthesis support beads to the attenuated total reflectance element.

Figure 1c is a detailed view of the a segment of the apparatus of Figure 1b, showing the optical relationship of the solid support beads to the attenuated total reflectance element.

Figure 2 illustrates a solid state Friedel-Crafts alkylation reaction that may be used in carrying out the present invention.

Figure 3 illustrates a Suzuki reaction.

Figure 4 illustrates a solid state Suzuki reaction that may be used in carrying out the present invention.

Figure 5 illustrates a Mitsunobu reaction.

Figure 6 illustrates a solid state Mitsunobu reaction that may be used in carrying out the present invention.

Figure 7 illustrates the results obtained with a Friedel-Crafts reaction of the present invention. The upper box of this figure is a waterfall plot of absorbance versus time; the lower box of this figure is a contour plot over time.

Figure 8 illustrates the wash steps carried out with the reaction of Figure 7, with a total of 15 washing steps being employed. The upper box of this figure is a waterfall plot of absorbance versus wash number. As can be seen there is a large initial absorbance with the trend towards zero absorbance as the washes progress. The bottom portion of the figure is a contour plot with the content of the wash indicated at the top of the plot and the wash progression proceeding from right to left. It can be seen from this plot that the first 5 washes remove a substantial amount of the excess reagent.

Figure 9 illustrates the results obtained with a Suzuki coupling reaction with the present invention.

Figure 10 illustrates the results for the first step of a Mitsunobu reaction in carrying out the present invention.

Figure 11 illustrates the monitoring of chemistry on beads, or the solid phase, and not the reaction solution, in a Suzuki coupling reaction in the present invention.

Figure 12 schematically illustrates a combinatorial library synthesis apparatus employing an attenuated total reflection monitor of the present invention.

Figure 13a illustrates a probe and support structure arrangement for monitoring reactions in a plurality of reaction wells.

Figure 13b illustrates a probe and support structure arrangement for monitoring reactions in a plurality of reaction wells.

Figure 13c illustrates a probe and support structure arrangement for monitoring reactions in a plurality of reaction wells.

Detailed Description of the Preferred Embodiments

The present invention is useful for all types of solid phase reactions, including batch reactions, single reactions, or reactions incorporated into a combinatorial synthesis process for making a combinatorial library. Combinatorial libraries are sets or collections of different compounds, which compounds are immobilized on a solid support, with different compounds immobilized on a different discrete solid support

as described below. The compounds may be oligomers or nonoligomers, as also discussed below.

In general, the chemical syntheses and reactions described in the Examples below can be monitored with the apparatus illustrated in **Figure 1a**. The apparatus comprises a fiber optic coupled attenuated total reflection (ATR) probe **20** connected to a ultraviolet/visible light spectrograph **25**. The ATR probe (obtained from Equitech International, Aiken, South Carolina, USA) was employed a three-bounce design attenuated total reflection element **22** and incorporated 400 μm fiber optic fibers as the cable interconnecting the spectrograph to the probe. Two different spectrographs were used for this work depending on the number of channels monitored: A single channel spectrograph using a deuterium light source (Zeiss MCS 501, Custom Sensors and Technology, St. Louis, Missouri, USA) and a four channel spectrograph using a xenon light source (Equitech International, Aiken, SC). The spectrograph was coupled to a personal computer **24** with a standard interface board for data analysis and display. We used the ASPECT™ data acquisition and display software, available from Zeiss, Germany, and also used LABVIEW™ data acquisition, display and analysis software, available from National Instruments, USA.

Attenuated total reflection elements, monitors and spectroscopy techniques are known and can be carried out in accordance with known techniques, such as described in U.S. Patents Nos. 5,814,565; 5,465,151; 5,070,243; and 3,752,584. In general, the total internal reflection can be carried out with ultraviolet light, near-ultraviolet light, visible light or infrared light. The attenuated total reflection element may be formed from any suitable material that is substantially optically transparent at the wavelength employed, but is generally formed from an inert insoluble inorganic crystal material such as sapphire, glass, quartz, germanium, zinc selenide, diamond (including diamond like carbon), and combinations thereof. The attenuated total reflection element may be formed from composites or layers in accordance with known optical fabrication techniques, and may uncoated or coated with a material that will facilitate adhesion of the solid supports to the attenuated total reflection element

In use, the probe **20** is inserted into a solid phase reaction medium **30** comprising a liquid phase **31** and discrete solid supports, preferably polystyrene beads, as the solid support **32**. As shown in **Figure 1b**, the solid particles **32** adhere or bind to the ATR element **22**, with light entering and leaving the element along

paths **35, 36**. As shown in **Figure 1c**, light **37** from the ATR element **22** is believed to enter and penetrate the solid supports **32** due to the adhesion or binding of the solid supports **32** to the ATR element **22**. Note that when the solid support is a separate discrete solid support such as a particle or bead, it is preferred that a major portion, or substantially all of, the exposed surface of the attenuated total reflection element is coated with a continuous layer of said separate discrete solid support that has a thickness of at least one of said separate discrete solid supports, as illustrated in **Figure 1b.**

Solid supports used to carry out the present invention are typically discrete solid supports. Discrete solid supports may be separate from one another as described above, or may be discrete regions on a surface portion of a unitary substrate. Such "chip-type" or "pin-type" solid supports are known. *See, e.g.,* U.S. Patent No. 5,288,514 to Ellman (pin-based support); U.S. Patent No. 5,510,270 to Fodor et al. (chip-based support) (the disclosures of all United States patent references cited herein are to be incorporated by reference herein in their entirety). Separate discrete supports such as particles or beads (these terms being used interchangeably herein), disks, fibers, needles or the like, are currently preferred.

The discrete solid supports are formed from a polymer such as polystyrene. In general, the solid substrates are beads, which may be completely solid throughout, porous, deformable or hard. The beads will generally be at least 10, 20 or 50 to 250, 500, or 2000 μm in diameter, and are most typically 50 to 250 μm in diameter. Any convenient composition can be used for the particles or beads, including cellulose, pore-glass, silica gel, polystyrene beads such as polystyrene beads cross-linked with divinylbenzene, grafted copolymer beads such as polyethyleneglycol/polystyrene, polyacrylamide beads, latex beads, dimethylacrylamide beads, composites such as glass particles coated with a hydrophobic polymer such as cross-linked polystyrene or a fluorinated ethylene polymer to which is grafted linear polystyrene, and the like. Where separate discrete solid supports such as particles or beads are employed, they generally comprise from about 1 to 99 percent by weight of the total reaction mixture.

The reaction medium apart from the solid support is, in general, a liquid. In general, the reaction medium will comprise from about 1 to 99 percent by weight of the total reaction medium (where the solid support is separate from the container in which the reaction mixture is held, as in the case of particles or beads). Any suitable

liquid may be employed, including aqueous liquids (water), nonaqueous liquids (*e.g.*, organic solvents), and combinations thereof or mixtures thereof. The liquid may be a single phase or multi-phase solution. Organic solvents used to carry out the present invention may be polar or nonpolar, protic or aprotic, etc. The particular solvent or mixture of solvents depends upon the particular reaction being carried out. Examples of suitable solvents for use in the reaction medium include, but are not limited to, methylene chloride, dimethyl formamide, 1-methyl-2-pyrrolidinone , methanol, ethanol, water, toluene, tetrahydrofuran, dioxane, ethyl acetate, chloroform, acetone, acetic anhydride, pentane, hexane, benzene, carbon tetrachloride, diethyl ether, acetonitrile, etc.

The reaction medium will include the reagents necessary to carry out the reaction desired, such as one or more reactants, a catalyst and/or initiator if necessary for the particular reaction, etc. All can be routinely determined by skilled artisans based on the particular reaction desired. During monitoring, the reaction mixture is held or placed in conditions that cause or permit the reaction to progress. The particular reaction conditions are not critical, depend upon the particular reaction desired, and can be routinely determined by skilled artisans. The reaction may be carried out under atmospheric pressure, elevated pressure, or reduced pressure; the reaction may be carried out at room temperature, elevated temperature, or reduced temperature; the reaction may be carried out at a neutral, acidic, or basic pH; the reaction may be carried out with or without an inert blanketing gas such as nitrogen or argon; etc.

Any suitable reaction can be employed to carry out the solid-phase synthesis that is monitored or observed by the present invention. *See, e.g.,* U.S. Patent No. 5,565,324. Specific examples of suitable reactions include, but are by no means limited to, Mitsunobu, Freidel-Crafts, Suzuki, Merrifield (for the synthesis of polypeptides)(*see, e.g.,* Merrifield, *J. Am. Chem. Soc.* 85, 2149 (1963); Merrifield, *Science* 150, 178 (1965), Hofmann, Grignard, Cannizarro, Clemmensen, Knovenagel, Perkin, Wittig, Wolff-Kishner, Ruff, Reimer-Tiemann, Kiliani-Fisher, Markonikov, Curtius, Lossen, Schoniger, Williamson, Bogert-Cook, and Hell-Volhard-Zellinsky reactions, etc.

The reaction or reactions employed in carrying out the present invention may thus be selected to produce a variety of products, the progression of the synthesis of

which products can be monitored as described herein. Such products are, in general, non-oligomers, oligomers, or combinations thereof.

Non-oligomer reaction products include a wide variety of organic molecules, such as heterocyclics, aromatics, alicyclics, aliphatics and combinations thereof, comprising steroids, antibiotics, enzyme inhibitors, ligands, hormones, drugs, alkaloids, opioids, terpenes, porphyrins, toxins, catalysts, as well as combinations thereof.

Oligomer reaction products include oligopeptides, oligonucleotides, oligosaccharides, polylipids, polyesters, polyamides, polyurethanes, polyureas, polyethers, and poly (phosphorus derivatives), *e.g.* phosphates, phosphonates, phosphoramides, phosphonamides, phosphites, phosphinamides, *etc.*, poly (sulfur derivatives) *e.g.*, sulfones, sulfonates, sulfites, sulfonamides, sulfenamides, *etc.*, where for the phosphorous and sulfur derivatives the indicated heteroatom for the most part will be bonded to C,H,N,O or S, and combinations thereof.

The present invention provides a direct monitoring technique rather than an indirect monitoring technique. By directly monitoring the progression of the reaction with the methods described herein, the reaction is advantageously monitored in real time as the reaction occurs. Thus the progression of the reaction need not be inferred from other measurements, and it is not necessary to have an intervening sampling, handling, or other processing step that intervenes between the reaction event or events and analysis. Further, the reaction need not be "opened" or otherwise disrupted as atmosphere, pressure, temperature, concentration, volume and/or stirring is changed by the sampling or processing step. As a result, individual solid state reactions can be efficiently and effectively monitored so that the reaction materials and equipment can be optimized for a particular combinatorial chemistry and synthesis program, allowing the time and effort required to carry out the combinatorial synthesis to be reduced.

It will be appreciated that, while the present invention is primarily concerned with monitoring the progression of synthesis reactions, and while the object of a series of reactions may be to synthesize a compound, that a given reaction may involve the addition, removal (*e.g.*, degradation), or substitution of a chemical unit from the core compound in solid phase, and that the progression of all such reactions may be monitored by the present invention.

The method of the present invention can be employed to monitor single batch reactions, as described in connection with Figure 1 above, or can be used to monitor sequential and/or concurrent reactions, such as employed in the synthesis of a combinatorial chemical library, where the reaction product may be oligomers or nonoligomers as described above. Such procedures are readily automated, and an apparatus for generating a solid phase combinatorial library while utilizing the methods of the present invention is schematically illustrated in **Figure 12.** The apparatus includes a chemical synthesis robot **101** configured to receive a plurality of reaction wells, as discussed in greater detail below. A reagent repository **102** is operatively associated with the chemical synthesis robot in accordance with standard techniques. An attenuated total reflection element as described above (illustrated in Figures 13a-13c) is operatively associated with the chemical synthesis robot for insertion into at least one, and preferably a multiplicity or even all of the reaction wells. An attenuated total reflection monitor **103** is connected to each attenuated total reflection element, which are configured to monitor a solid phase synthesis reaction in the corresponding reaction well by attenuated total reflection spectroscopy. A synthesis controller **104** is operatively associated with both the reagent repository and the chemical synthesis robot. The synthesis controller is configured through the implementation of hardware and/or software instructions to control the construction of a solid phase combinatorial library in the plurality of reaction wells. Synthesis robots, reagent repositories and controllers therefore are known in the art and can be implemented in accordance with standard techniques. *See, e.g.,* U.S. Patent No. 5,463,564 to Agrafiotis et al (the disclosures of all United States patent references cited herein are to be incorporated herein by reference). The synthesis controller is operatively associated with the attenuated total reflection monitor through an appropriate interface, with the synthesis controller configured through hardware and/or software instructions to end a particular synthetic step or reaction upon detection of completion of a chemical reaction, so that the overall combinatorial synthesis scheme can proceed to the next cycle of reaction steps.

The reaction wells may be incorporated into the robot, but are typically provided in a separate structure that is insertable into or carried by the robot, as is known in the art. Microtiter plates, individual containers, columns, gels, Teraski plates, flasks, Merrifield synthesis vessels, etc., can be employed. Preferred are

structures that contain a plurality of reaction wells, such as microtiter plates, which typically contain from 96 up to 2,304 reaction wells, or more. Filter plates that match the support structure can be used to drain the liquid contents of the reaction well while retaining the solid supports therein, in accordance with known techniques.

The attenuated total reflection element can be associated with the reaction wells in a combinatorial synthesis apparatus as described in Figure 12 in any of a variety of ways, some of which are illustrated in Figures 13a-c. In **Figure 13a**, the well support structure **120a** contains a plurality of reaction wells **121a**. The bottom of each well is plugged and sealed by a probe **122a** that terminates with the attenuated total reflection element **123a**. The probes are thus connected to the robot stage with the support structure removably connected thereto, and with each well sealed by the reflection element. The contents of each well can be pipetted therefrom for subsequent reactions.

An alternate approach is illustrated in **Figure 13b**, which again comprises a support structure **120b** containing a plurality of reaction wells **121b**. Here, however, the total reflection elements **123b** are rigidly and sealably connected into the support structure **120b**, while the probe elements **122b** are removably contacted to the reflection elements. Thus the entire support structure can be simply secured through an appropriate stage to the probe elements, which again are connected to the synthesis robot.

Still another approach is illustrated in **Figure 13c**, which again comprises a support structure **120c** having a plurality of reaction wells **121c**, with a plurality of probes **122c**, each carrying an attenuated total reflection element **123c**, inserted into each reaction well. This structure can employ a conventional microtiter plate. The probes are carried by the synthesis robot, which can be structured so that the probes are placed in the reaction well before, during, or after the addition of supports, reaction medium or liquid, and other reagents into each well.

In use, the foregoing apparatus provides a method of making a combinatorial library by solid phase chemical synthesis carried out by the following steps:

(a) combining (typically in a reaction well) a solid support, a liquid reaction medium and reaction reagents to produce a reaction mixture;

(b) contacting an attenuated total reflection element to the reaction mixture; then

(c) monitoring the chemical reaction on the solid support through the attenuated total reflection element to thereby detect completion of said reaction, then

(d) separating the solid support from said liquid reaction medium and said reaction reagents upon detecting completion of said reaction; and then

(e) repeating steps *(a)* through *(d)* with the separated solid supports produced in step *(d)* above.

The repeating step is carried out cyclically until the desired number of compounds is produced as the combinatorial library. Again, the library so produced may be comprised of oligomer or nonoligomer compounds, as described above, by any type of solid phase reaction, including but not limited to those described above.

In the context of combinatorial chemistry and the present invention, completion of the reaction is achieved when the reaction is sufficiently complete to achieve reasonable uniformity of the product population for a given library constituent, or group of constituents, to achieve the desired result of producing a useful combinatorial library. Preferably, completion of the reaction occurs when the reaction reaches equilibrium.

Typically, the repeating of steps *(a)* through *(d)* above is carried out with a different reagent or substrate, or any other technique useful for generating a diverse combinatorial library. For example, with the apparatus illustrated in **Figures 13a-13c**, steps *(a)* through *(d)* above are concurrently carried out in a plurality of different reaction wells, and then a new set initiated in step *(e)*, again typically in a plurality of different reaction wells. The steps *(a)* through *(d)* may be cyclically repeated, individually or as sets of concurrent reactions, as many times as necessary to produce the desired combinatorial library, typically 2, 3 or 4 repetitions up to 10, 20, 30 or 40 repetitions or more.

Depending upon the particular apparatus employed, each reaction cycle may be concurrently carried out in 10, 20 or 30 up to 400, 600 or 800 or more wells or reaction vessels. Current formats include 96 well and 384 well microtiter plates, but it is not necessary that every well in the plate be used.

It will be appreciated that diversity in the library can be achieved by any means of solid phase or solid state synthesis, such as by carrying out different series of syntheses in different wells, by pooling the solid supports from multiple different

reaction wells (including some or all) after a synthetic cycle, splitting that pool back into different reaction wells for a further set of reactions, and then repeating that cycle, in accordance with known techniques. *See, e.g.,* U.S. Patents No. 5,656,324 to Still et al.; PCT Application No. WO97/37953 to Geysen et al. Reactions, reaction conditions and reaction products include but are not limited to those described above. By optimizing a plurality of the reaction cycles (or even a majority of or all of the reaction cycles) through the application of the monitoring steps described herein, combinatorial libraries containing at least 100, 500, 1,000 or 5,000 different compounds up to 10,00, 20,000, 50,000, 100,000, up to 10^6 different compounds or more, can be produced more rapidly than by prior techniques. Depending upon the intended purpose of the combinatorial library the library may be relatively large or relatively small, but the present invention is useful in the preparation of both relatively small libraries and relatively large libraries on a more expeditious basis.

The process of synthesizing a combinatorial library may be varied from that described above. In general, when the synthesis of a combinatorial library is carried out, a plurality of, more preferably a majority of, and most preferably all of the reaction cycles are terminated upon completion of the chemical reaction being carried out in that cycle. Completion can be determined by attenuated total reflection spectroscopy as described above. In a particular library synthesis procedure, the attenuated total reflection spectroscopy step may be carried out *in situ* during the chemical reaction cycle of interest, and completion directly detected. In the alternative, the attenuated total reflection spectroscopy step may be carried out *a priori* on a model reaction system prior to the chemical reaction cycles of interest to, the reaction optimized and a necessary reaction time determined. Then, during the combinatorial library synthesis, the reaction cycle that incorporates a reaction step based upon the reaction model (and for which a reaction time has been determined *a priori*) may be terminated upon completion of the previously determined reaction time. In a particular combinatorial synthesis, a plurality of, a majority of, or all of the reaction cycles may be carried out to completion, with completion being determined by either *in situ* measurement or *a priori* measurement as described above, or combinations thereof. It will also be appreciated that not every reaction in a particular combinatorial synthesis need by optimized by the techniques described herein, so long as the overall library synthesis time is advantageously reduced. Further, where a time

to completion is determined *a priori* from a model reaction, that model reaction can be the same as the reaction carried out in the synthesis of the combinatorial library, or may differ in one or more parameters, with the reaction time for the combinatorial synthesis reaction being interpolated from the model reaction. When a time to completion is interpolated from a different reaction, it is preferably interpolated from a plurality of different reactions in accordance with known techniques.

In general, by use of the present invention in conjunction with making a combinatorial library, the time to synthesize the library can be substantially reduced. In general, all of the reaction cycles may be completed in a time that averages not greater than 6, 8 or 10 hours per reaction cycle (typically, an average time of at least 1 or 2 hours per reaction cycle is employed). Thus, for example, when a library is synthesized in three consecutive reaction cycles, the cycles having reaction times of 2, 4 and 6 hours respectively, the average reaction time per cycle is 4 hours. Note that in determining the average time any resting time or periods of inactivity between cycles is not counted, but the time for all aspects of each reaction, including mixing and washing steps, is included.

In combinatorial syntheses, the compounds on the solid supports or the solid supports themselves may be tagged for later decoding by any suitable technique in accordance with known procedures, including but not limited to oligonucleotide tags and oligopeptide tags, *see, e.g.,* S. Brenner and R. Lerner, *Proc. Natl. Acad. Sci. USA* **89**, 5381 (1992); J. Kerr et al., *J. Am. Chem. Soc.* **115**, 2529 (1993), nonsequential tags, such as described in U.S. Patent No. 5,565,324 to Still, or mass-based encoding such as described in PCT Application WO97/37953 to Geysen et al. Tagging reactions may be carried out concurrently with the reactions employed to synthesis the members of the combinatorial library in the same reaction medium.

The present invention is explained in greater detail in the following non-limiting examples.

EXAMPLE 1

Solid State Friedel-Crafts Reactions

In general, a Friedel-Crafts reaction is an electrophilic substitution The general reaction steps are as follows (r. Morrison and R. Boyd, Organic Chemistry (Allyn and

Bacon, Inc., 1973): The electrophile is typically a carbonium ion and is formed in an acid-base equilibrium, as illustrated in **Scheme 1** below:

Scheme 1

(1) $RCl + AlCl_3 \Leftrightarrow AlCl_4^- + R^{\oplus}$

(2) $R^{\oplus} + C_6H_6 \Leftrightarrow C_6H_5^{\oplus}(H)(R)$

(3) $C_6H_5^{\oplus}(H)(R) + AlCl_4^- \Leftrightarrow C_6H_5R + HCl + AlCl_3$

For the example discussed herein the chemistry involved is more particularly illustrated **Figure 2.** In brief, in an illustrative reaction, Biorad S-X1 resin, 5.0 grams, is suspended in 200 mL of methylene chloride to which N-chloromethyl phthalimide, 6.85 grams, is added and stirred. Solid iron is added, 0.8 grams, and the reaction is stirred at room temperature.

Example 2

Solid State Suzuki Reactions

Suzuki couplings involve the palladium-catalyzed couplings of unsaturated halides with boronic acids or esters. Suzuki coupling reactions are known in the art. *See, e.g.,* G. Smith et al., *J. Org. Chem.* 59, 8151 (1994). The general Suzuki coupling is shown in **Figure 3.** An example of this type of reaction applied to solid phase synthesis is shown in **Figure 4.** In brief, in an illustrative reaction, precursor resin, 0.4 grams, is suspended in 6 mL of methylene chloride and stirred. To this phenylboronic acid, 0.2.4 grams, 2M sodium carbonate solution, 0.95 mL, and tetrakis(triphenyl phosphine) palladium, 44 milligrams, are added and refluxed.

EXAMPLE 3

Solid State Mitsunobu Reactions

Mitsunobu reactions refer to the reaction of hydroxy compounds with acids ($pK_a \leq 11$) in the presence of triphenylphosphine and diethyl azodicarboxylate. *See, e.g.,* M. Varasi et al., *J. Org. Chem.* **52**, 4235 (1987). This scheme is used for the functionalization of alcohols and related compounds. The general Mitsunobu reaction is shown in **Figure 5**. A specific solid phase Mitsunobu reaction is shown in **Figure 6**. In brief, in an illustrative reaction, Sasrin resin, 2.16 grams, is suspended in 50 mL of tetrahydorfuran and gently agitated for 30 minutes under a blanket of nitrogen. N-hydroxyphthalimide, 1.60 grams, and triphenylphosphine, 2.52 grams, is added and the mixture is agitated until these reagents are dissolved. Diisopropyl azodicarboxylate, 2.0 mL, is added and the mixture stirred at room temperature.

EXAMPLE 4

Monitoring of a Solid State Friedel-Craft Reaction

The spectral results for a Friedel-Craft reaction, step 1, as described above as monitored by an apparatus described in Figures 1a-c above, are presented in **Figure 7**. UV-vis data are displayed as waterfall and contour plots. Note the increase in absorbance over time. It was found that the reaction finished quite quickly. In addition, the actual number of washes specified in the literature to complete the reaction procedure was discovered to be in excess of what is truly required to clean the beads. The typical wash cycle called for 15 washes; by monitoring the wash cycle through this number of washes, as shown in **Figure 8**, it became is clear that fewer washes were sufficient.

EXAMPLE 5

Monitoring of a Solid State Suzuki Reaction

Results from the Suzuki coupling reaction shown in **Figure 4**, as monitored by an apparatus described in Figures 1a-c, are presented in **Figure 9**. As in Example 4 above, again note the increase in absorbance over time during the progression of the reaction.

EXAMPLE 6

Monitoring of a Solid State Mitsunobu Reaction

The results for the first step of the Mitsunobu reaction described above, as monitored by an apparatus as described in Figures 1a-c, are shown in **Figure 10**. Again, note the increase in absorbance over time during the progression of the reaction.

EXAMPLE 7

Direct Monitoring of Solid Phase Chemistry

Figure 11 illustrates that the present invention directly monitors the synthesis on the beads, and not indirectly through the reaction medium. Again, an apparatus as described in Figures 1a-c was employed. The "last reaction spectrum" (middle trace) is typical of the waterfall traces collected during the reaction. The probe was removed from the reaction vessel and allowed to air dry. A thin film of residue, determined to be beads, was observed on the sapphire probe tip. The spectrum collected after removal of the probe from solution is slightly shifted due to drying, but closely matches that of the reaction spectrum. (As it was weaker than that of the reaction scan, it is plotted at two times the actual intensity.) Next, the probe was wiped clean with a KIMWIPE™ paper. No spectral response was observed in the spectrum of the wiped probe. These data show that the increase in absorbance over time, as described in Examples 4 to 6 above, represents a direct monitoring of the synthesis reaction.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method for monitoring a solid phase chemical reaction, said method comprising the steps of:

providing a reaction mixture comprising a solid support and a liquid reaction medium,

contacting an attenuated total reflection element to said reaction mixture; and then

directly monitoring said chemical reaction on said solid support through said attenuated total reflection element;

wherein said monitoring step is carried out by attenuated total reflection spectroscopy.

2. A method according to claim 1, wherein:

said contacting step is carried out under conditions that cause the progression of said reaction;

said monitoring step is carried out by measuring light absorbance by said reaction mixture; and

changing light absorbance is positively associated with the progression of said reaction.

3. A method according to claim 2, wherein said reaction is a synthesis reaction, and wherein increasing light absorbance is positively associated with the progression of said reaction.

4. A method according to claim 2, wherein said reaction is a degradation reaction, and wherein decreasing light absorbance is positively associated with the progression of said reaction.

5. A method according to claim 1, wherein said directly monitoring step is a continuous directly monitoring step.

6. A method according to claim 1, wherein said reaction is a Mitsunobu reaction.

7. A method according to claim 1, wherein said reaction is a Freidel-Craft reaction.

8. A method according to claim 1, wherein said reaction is a Suzuki reaction.

9. A method according to claim 1, wherein said solid support comprises a plurality of discrete solid supports.

10. A method according to claim 1, wherein said solid support comprises a plurality of separate discrete solid supports.

11. A method according to claim 1, wherein said solid support is formed of polystyrene.

12. A method according to claim 1, wherein said discrete solid support is bound to said attenuated total reflection element.

13. A method according to claim 1, wherein said attenuated total reflection element is formed from a material selected from the group consisting of sapphire, glass, quartz, germanium, zinc selenide, silicon, diamond, and combinations thereof.

14. An apparatus for generating a solid phase combinatorial library, said apparatus comprising:

a chemical synthesis robot configured to receive a plurality of reaction wells;

a reagent repository operatively associated with said chemical synthesis robot;

an attenuated total reflection element operatively associated with said chemical synthesis robot for insertion into at least one of said reaction wells;

an attenuated total reflection monitor operatively associated with said attenuated total reflection element and configured to monitor a solid phase synthesis reaction in said reaction well by attenuated total reflection spectroscopy; and

a synthesis controller operatively associated with both said reagent repository and said chemical synthesis robot; said synthesis controller configured to control the construction of a solid phase combinatorial library in said plurality of reaction wells;

with said synthesis controller operatively associated with said attenuated total reflection monitor.

15. An apparatus according to claim 14, further comprising a support structure carried by said synthesis robot, said support structure containing said plurality of reaction wells.

16. An apparatus according to claim 15, wherein said support structure is a microtiter plate.

17. An apparatus according to claim 14, wherein said solid support comprises a plurality of discrete solid supports.

18. An apparatus according to claim 14, wherein said solid support comprises a plurality of separate discrete solid supports.

19. An apparatus according to claim 14, wherein said solid support is formed of polystyrene.

20. An apparatus according to claim 14, wherein said discrete solid support is bound to said attenuated total reflection element.

21. An apparatus according to claim 14, wherein said attenuated total reflection element is formed from a material selected from the group consisting of sapphire, glass, quartz, germanium, zinc selenide, silicon, diamond, and combinations thereof.

22. A method of making a combinatorial library by solid phase chemical synthesis, said method comprising the steps of:

(a) combining in a reaction well a solid support, a liquid reaction medium and reaction reagents to produce a reaction mixture;

(b) contacting an attenuated total reflection element to said reaction mixture; then

(c) directly monitoring said chemical reaction on said solid support through said attenuated total reflection element, wherein said monitoring step is carried out by attenuated total reflection spectroscopy, to detect completion of said reaction; then

(d) separating said solid support from said liquid reaction medium and said reaction reagents upon detecting said completion of said reaction; and then

(e) repeating steps *(a)* through *(c)* with said separated solid supports produced in step *(d)* above.

23. A method according to claim 22, wherein steps *(a)* through *(e)* are concurrently repeated in a plurality of separate reaction wells.

24. A method according to claim 22, wherein said directly monitoring step is a continuous directly monitoring step.

25. A method according to claim 22, wherein said reaction is a Mitsunobu reaction.

26. A method according to claim 22, wherein said reaction is a Freidel-Craft reaction.

27. A method according to claim 22, wherein said reaction is a Suzuki reaction.

28. A method according to claim 22, wherein said solid support comprises a plurality of discrete solid supports.

29. A method according to claim 22, wherein said solid support comprises a plurality of separate discrete solid supports.

30. A method according to claim 22, wherein said solid support is formed of polystyrene.

31. A method according to claim 22, wherein said discrete solid support is bound to said attenuated total reflection element.

32. A method according to claim 22, wherein said attenuated total reflection element is formed from a material selected from the group consisting of sapphire, glass, quartz, germanium, zinc selenide, silicon, diamond, and combinations thereof.

33. In a method of making a combinatorial library comprising at least 100 different compounds, with each of said compounds immobilized on a discrete solid support, by at least two sequential reaction cycles, and with each of said reaction cycles comprising a solid phase chemical reaction, the improvement comprising:

completing all of said sequential reaction cycles in a total time that averages from 1 to 8 hours for each of said sequential reaction cycles.

34. A method according to claim 33, wherein a plurality of said sequential reaction cycles are terminated upon completion of the corresponding solid phase chemical reaction.

35. A method according to claim 34, wherein completion of said plurality of sequential reaction cycles is determined by attenuated total reflection spectroscopy.

36. A method according to claim 35, wherein said attenuated total reflection spectroscopy is carried out by:

(a) combining a solid support, a liquid reaction medium and reaction reagents to produce a reaction mixture;

(b) contacting an attenuated total reflection element to said reaction mixture; and then

(c) directly monitoring said chemical reaction on said solid support through said attenuated total reflection element, wherein said monitoring step is carried out by attenuated total reflection spectroscopy, to detect completion of said reaction.

37. A method according to claim 36, wherein said attenuated total reflection spectroscopy is carried out *in situ* during each of said plurality of chemical reaction cycles.

38. A method according to claim 36, wherein said attenuated total reflection spectroscopy is carried out *a priori* on a model reaction prior to each of said plurality of chemical reaction cycles.

39. A method according to claim 33, wherein each of said reaction cycles is concurrently repeated in at least 10 separate reaction wells.

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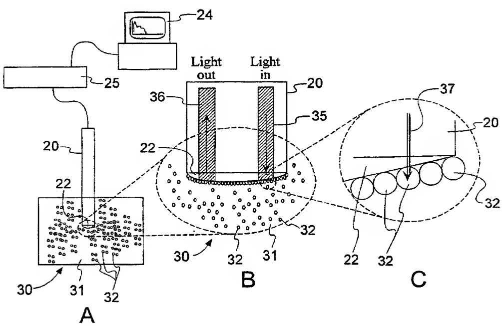
[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS



(57) Abstract: A method for monitoring a solid phase chemical reaction comprises the steps of: *(a)* providing a reaction mixture comprising a solid support and a reaction medium, *(b)* contacting an attenuated total reflection element to said reaction mixture; and then *(c)* monitoring the chemical reaction on the solid support through the attenuated total reflection element. The monitoring step is carried out by attenuated total reflection spectroscopy. An advantage of the invention is that the chemical reaction on the solid support may be directly monitored, rather than indirectly monitoring that chemical reaction by monitoring reaction constituents in the reaction medium.

WO 01/29537 A3

FIG. 1A

FIG. 1B

FIG. 1C

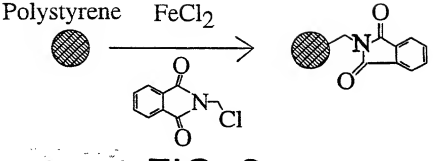


FIG. 2

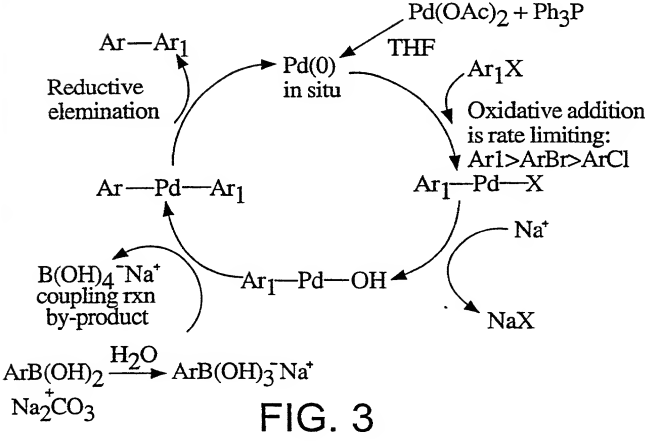


FIG. 3

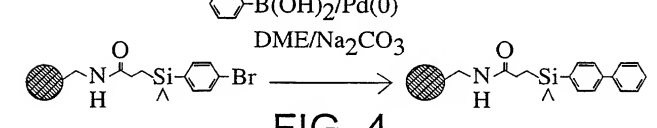
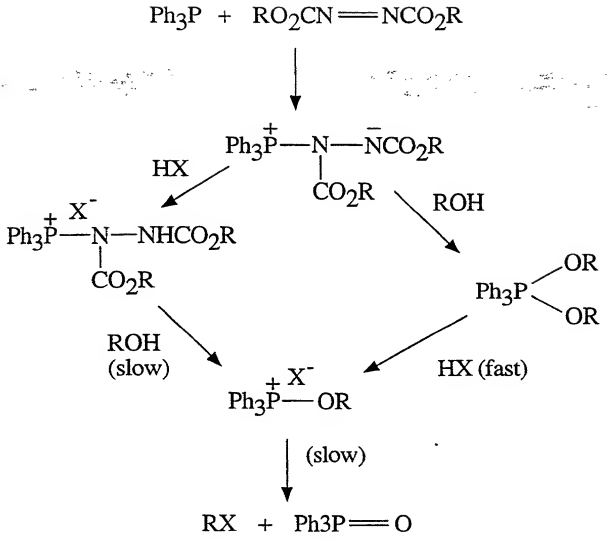


FIG. 4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Ph3P+—N—N−CO2R
CO2R
HX
ROH
Ph3P+—N—NHCO2R
X-
CO2R
Ph3P
OR
OR
ROH
(slow)
HX (fast)
Ph3P+—OR
X-
(slow)
RX + Ph3P=O

FIG. 5

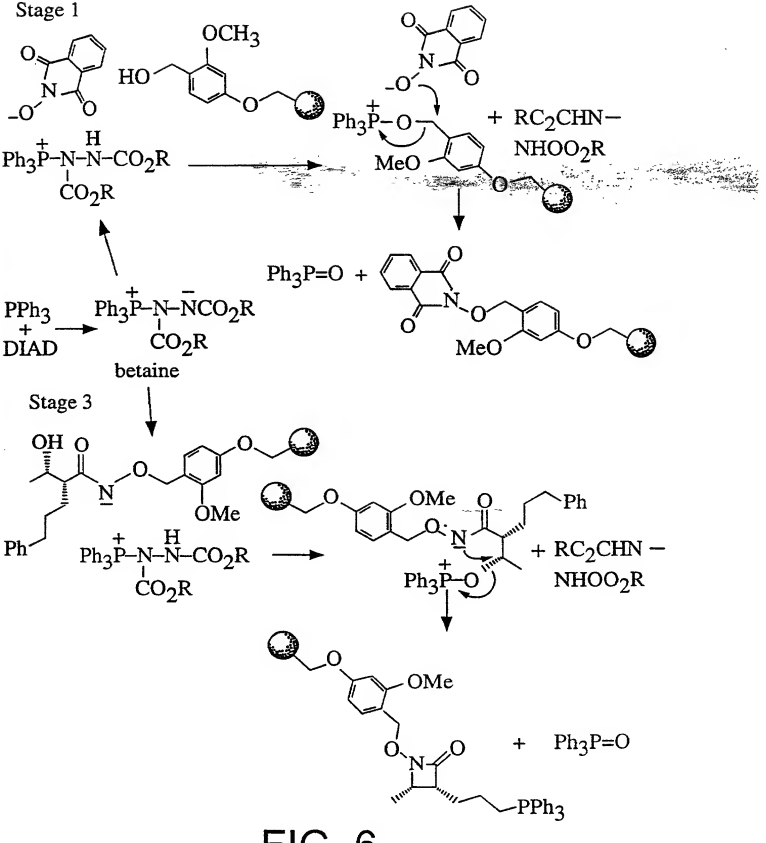
Stage 1
OCH3
HO
+ RC2CHN–
NHOO2R
MeO
Ph3P=O +
MeO
PPh3
+
DIAD
betaine
Stage 3
OH
OMe
Ph
+ RC2CHN –
NHOO2R
OMe
+ Ph3P=O
PPh3

FIG. 6

Absorbance
1
0.5
0
700
600
500
400
300
200
nm
Time (min)
250
200
150
100
50
450
400
350
300
250
200
nm
50
100
150
200
250
Time (min)

FIG. 7

Washing step for step 1
Absorbance
Wash number
Dioxan
Dioxan & Acid
nm
Dioxan
MeOH
DCM
Wash number

FIG. 8

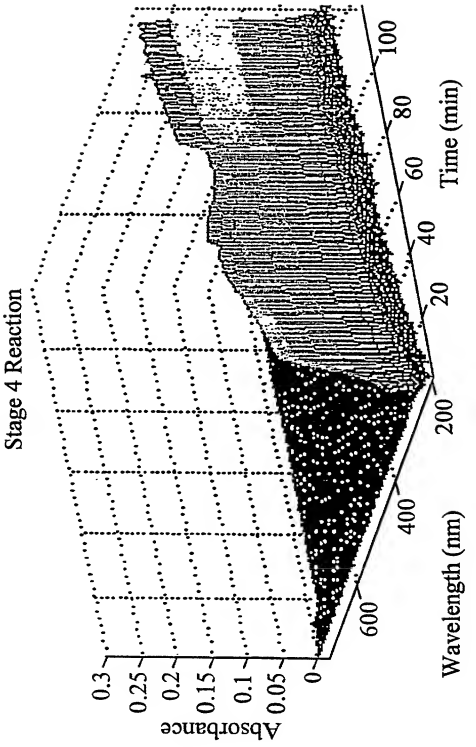
Stage 4 Reaction
Absorbance
0.3
0.25
0.2
0.15
0.1
0.05
0
Wavelength (nm)
600
400
200
Time (min)
20
40
60
80
100

FIG. 9

Absorbance
0
0.5
1
1.5
2
Wavelength (nm)
700
600
500
600
300
200
Time (min)
10
20
30
40
50
60

FIG. 10

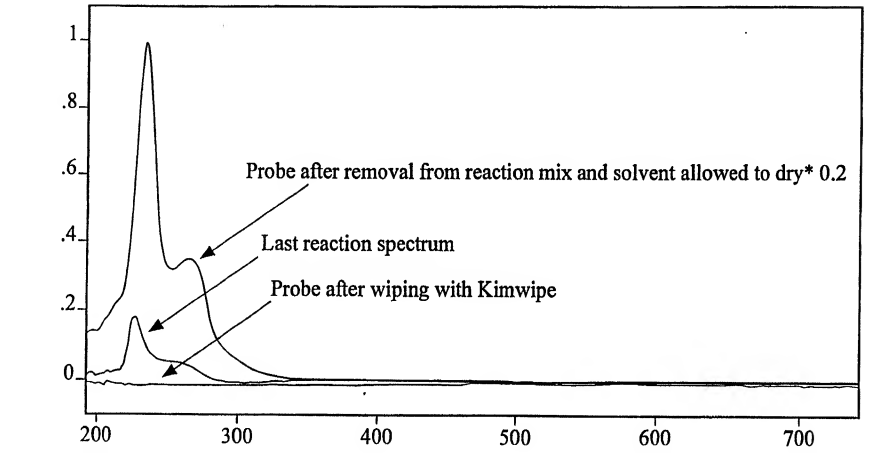
1
.8
.6
.4
.2
0
Probe after removal from reaction mix and solvent allowed to dry* 0.2
Last reaction spectrum
Probe after wiping with Kimwipe
200
300
400
500
600
700
Absorbance/Nanometers
Overlay-X-Zoom CURSOR

FIG. 11

104
Synthesis Controller
102
Reagent Repository
101
Chemical Synthesis Robot
103
Attenuated Total Reflection Monitor

FIG. 12

FIG. 13A

FIG. 13B

FIG. 13C

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☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

SIGNATURE of Applicant or Assignee of Record

Name	Joanne Elizabeth Anderson
Signature	Joanne E. Anderson
Date	22 Feb 2002

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	First Named Inventor	Anderson
	COMPLETE IF KNOWN	
	Application Number	/
	Filing Date	
	Group Art Unit	
	Examiner Name	

☐ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e))

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS

the specification of which *(Title of the Invention)*

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I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign		Foreign Filing Date	Priority	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/159,673	10/15/1999	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR : ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Joanne, Elizabeth | Family Name or Surname ANDERSON

Inventor's Signature Joanne E. Anderson | Date 22 Feb 2002

Residence: City Durham | State NC | Country US | Citizenship US

Mailing Address GlaxoSmithKline

Mailing Address Five Moore Drive, PO Box 13398

City Research Triangle Park | State NC | ZIP 27709 | Country US

NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Frank, Joseph | Family Name or Surname TARCZYNSKI

Inventor's Signature | Date

Residence: City Durham | State NC | Country US | Citizenship US

Mailing Address GlaxoSmithKline

Mailing Address Five Moore Drive, PO Box 13398

City Research Triangle Park | State NC | ZIP 27709 | Country US

☒ Additional inventors are being named on 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:		☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle [if any])		Family Name or Surname		
Dwight, Sherod		WALKER		
Inventor's Signature				Date
Residence: City	Durham	State NC	Country US	Citizenship US
Mailing Address	GlaxoSmithKline			
Mailing Address	Five Moore Drive, PO Box 13398			
City	Research Triangle Park	State NC	ZIP 27709	Country US

Name of Additional Joint Inventor, if any:		☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle [if any])		Family Name or Surname		
Inventor's Signature				Date
Residence: City		State	Country	Citizenship
Mailing Address				
Mailing Address				
City		State	ZIP	Country

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Given Name (first and middle [if any])		Family Name or Surname		
Inventor's Signature				Date
Residence: City		State	Country	Citizenship
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Mailing Address				
City		State	ZIP	Country

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POWER OF ATTORNEY OR AUTHORIZATION OF AGENT

Application Number	
Filing Date	
First Named Inventor	Anderson
Group Art Unit	
Examiner Name	
Attorney Docket Number	PU3703USW

I hereby appoint:

☒ Practitioners at Customer Number 23347 →

OR

☐ Practitioner(s) named below:

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Name	Registration Number

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

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☒ The above-mentioned Customer Number.

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I am the:

☒ Applicant/Inventor.

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

SIGNATURE of Applicant or Assignee of Record

Name	Frank, Joseph Tarczynski
Signature	
Date	22 Feb-2002

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 3 forms are submitted.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e))

Attorney Docket Number	PU3703USW
First Named Inventor	Anderson
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS

the specification of which *(Title of the Invention)*

☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 10/12/2000 as United States Application Number or PCT International Application Number PCT/US00/28218 and was amended on (MM/DD/YYYY) ☐ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign		Foreign Filing Date	Priority	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/159,673	10/15/1999	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR : ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Joanne, Elizabeth | Family Name or Surname ANDERSON

Inventor's Signature | Date

Residence: City Durham | State NC | Country US | Citizenship US

Mailing Address GlaxoSmithKline

Mailing Address Five Moore Drive, PO Box 13398

City Research Triangle Park | State NC | ZIP 27709 | Country US

NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Frank, Joseph | Family Name or Surname TARCZYNSKI

Inventor's Signature Frank Joseph Tar | Date 21 Feb 2002

Residence: City Durham | State NC | Country US | Citizenship US

Mailing Address GlaxoSmithKline

Mailing Address Five Moore Drive, PO Box 13398

City Research Triangle Park | State NC | ZIP 27709 | Country US

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ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:		☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle [if any])		Family Name or Surname		
Dwight, Sherod		WALKER		
Inventor's Signature				Date
Residence City	Durham	State NC	Country US	Citizenship US
Mailing Address	GlaxoSmithKline			
Mailing Address	Five Moore Drive, PO Box 13398			
City	Research Triangle Park	State NC	ZIP 27709	Country US

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Application Number	
Filing Date	
First Named Inventor	Anderson
Group Art Unit	
Examiner Name	
Attorney Docket Number	PU3703USW

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☐ Practitioner(s) named below:

Name	Registration Number

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

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Country			
Telephone		Fax	

I am the:

☒ Applicant/Inventor.

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96).

SIGNATURE of Applicant or Assignee of Record

Name	Dwight, Sherod Walker
Signature	
Date	02/08/02

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 3 forms are submitted.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	PU3703USW
	First Named Inventor	Anderson
	COMPLETE IF KNOWN	
	Application Number	/
	Filing Date	
	Group Art Unit	
	Examiner Name	

☐ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e))

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD AND APPARATUS FOR MONITORING SOLID PHASE CHEMICAL REACTIONS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 10/12/2000 as United States Application Number or PCT International Application Number PCT/US00/28218 and was amended on (MM/DD/YYYY) _____ (if applicable)

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			☐	☐	☐
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Given Name (first and middle [if any]) Joanne, Elizabeth | Family Name or Surname ANDERSON

Inventor's Signature | Date

Residence: City Durham | State NC | Country US | Citizenship US

Mailing Address GlaxoSmithKline

Mailing Address Five Moore Drive, PO Box 13398

City Research Triangle Park | State NC | ZIP 27709 | Country US

NAME OF SECOND INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) Frank, Joseph | Family Name or Surname TARCZYNSKI

Inventor's Signature | Date

Residence: City Durham | State NC | Country US | Citizenship US

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Page 1 of 1

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Given Name (first and middle [if any])		Family Name or Surname	
Dwight, Sherod		WALKER	
Inventor's Signature			Date 02/28/02
Residence: City	Durham	State NC	Country US	Citizenship US
Mailing Address	GlaxoSmithKline			
Mailing Address	Five Moore Drive, PO Box 13398			
City	Research Triangle Park	State NC	ZIP 27709	Country US

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Mailing Address				
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Mailing Address				
City		State	ZIP	Country